HEAD MOP Protocol Update: V5.20.2025

Section	Summary of Changes
4.3	Updated Biofluid Collection Chart Summary
4.3.2	Deleted Biofluid Collection Chart for 18 Month Visit (*combined Baseline and 18 Month in section 4.3.1*)
6.1.1	Updated Collection and Aliquot Tube Label format
6.1.1	Updated EDTA tube picture with label
6.5	Updated EDTA Baseline Blood Collection Schematic to inspect and identify tube expiration date
6.6	Added 18 Month EDTA Blood Collection Schematic to inspect and identify tube expiration date
General	Updated format throughout the document

Head-to-head Evaluation of tau tracers in Alzheimer's Disease

in collaboration with

The National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD)

Biofluid Collection, Processing and Shipment

Manual of Procedures

Version 3.0

May 2025

Table of Contents

1.0 ABBREVIATIONS

AD	Alzheimer's Disease
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra-acetic Acid
HEAD	Head-to-head Evaluation of tau tracers in Alzheimer's Disease
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
NCRAD	National Centralized Repository for Alzheimer's Disease and Related Dementias
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute
SST	Serum Separator Tube
UPS	United Parcel Service

2.0 PURPOSE

The collection of biofluids is an important part of the Head-to-head Evaluation of tau tracers in Alzheimer's Disease (HEAD) Study. The purpose of this manual is to provide study staff (PIs, study coordinators, phlebotomists) at the various study sites with instructions for collection and submission of biological samples for HEAD study visits. It includes instructions for biofluid submission to NCRAD located in Indianapolis at Indiana University.

The following samples will be sent to NCRAD:

- Serum
- Plasma
- Buffy Coat (DNA Extraction)
- Whole Blood for banking
- Whole Blood for RNA

This manual includes instructions for collection of blood, fractionation of blood from collection tubes, aliquoting, labeling, storage prior to shipping, and shipping to NCRAD.

These procedures are relevant to all study personnel responsible for processing specimens being provided to NCRAD for the HEAD protocol.

3.0 NCRAD INFORMATION

3.1 NCRAD Contacts

Tatiana Foroud, PhD, NCRAD Leader
Phone: 317-274-2218

Kelley Faber, MS, CCRC, Project Manager
Phone: 317-274-7360
Email: kelfaber@iu.edu

Diont'e Keys, BS, CCRP Study Coordinator
Phone: 317-274-7546
Email: dlkeys@iupui.edu

General NCRAD Contact Information
Phone: 1-800-526-2839
Alternate phone number: 317-278-8413
Email: alzstudy@iu.edu
Website: https://ncrad.org/
HEAD Study Specific Webpage: https://ncrad.org/coordinate-studies/head

Sample Shipment Mailing Address
HEAD at NCRAD
Indiana University School of Medicine
351 West 10th Street
TK-217
Indianapolis, IN 46202

3.2 NCRAD Hours of Operation

Indiana University business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Ambient samples must be shipped **Monday-Thursday only.**

Frozen samples must be shipped **Monday-Wednesday only**.

For packing and shipment details of samples, please refer to Section 9.0 of this protocol.

Check weather report to make sure impending weather events (blizzards, hurricanes, etc.) will not affect the shipping or delivery of the samples.

3.3 Holiday Schedules

- Please note that courier services may observe a different set of holidays. Please be sure to verify shipping dates with your courier prior to any holiday.

3.4 Holiday Observations

Date	Holiday
January 1	New Year's Day
3rd Monday in January	Martin Luther King, Jr Day
4th Monday in May	Memorial Day
June 19	Juneteenth (observed)
July 4	Independence Day (observed)
1st Monday in September	Labor Day
4th Thursday in November	Thanksgiving
4th Friday in November	Friday after Thanksgiving
December 25	Christmas Day
December 26-31	Winter Break

Please note that between December 23rd and January 3rd, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 6th. If at all possible, biological specimens for submission to Indiana University should **NOT** be collected and shipped to Indiana University after the second week of December. Should it be necessary to ship blood samples for DNA extraction to Indiana University during this period, please contact the Indiana University staff before December 20th by e-mailing alzstudy@iu.edu, so that they can arrange to have staff available to process incoming samples. **Please see:** https://www.ncrad.org/contact/holiday-closures/ **for additional information.**

- Please note that courier services may observe a different set of holidays.
- Please be sure to verify shipping dates with your courier prior to any holiday.
- **Weekend/holiday delivery must be arranged in advance with NCRAD staff.**

4.0 NCRAD Laboratory Collection

4.1 Site Required Equipment

The following materials and equipment are necessary for the processing of specimens at the collection site and are to be **supplied by the local site**:

- Personal Protective Equipment: lab coat, nitrile/latex gloves, safety glasses
- Tourniquet
- Alcohol Prep Pad
- Gauze Pad
- Bandage
- Butterfly needles and hub
- Microcentrifuge tube rack
- Sharps bin and lid
- Wet Ice Bucket
- Wet ice
- Pelleted dry ice

In order to process samples consistently across all projects and ensure the highest quality samples possible, project sites must have access to the following equipment:

- Centrifuge capable of ≥ 2000 x g with refrigeration to 4°C
- -80°C Freezer

In order to ship specimens, you must provide:

- Pelleted dry ice (approximately 45 lbs per shipment)

4.2 Biospecimens Sent to NCRAD

Samples are to be submitted according to the shipping methods outlined in Section 9.0. Guidelines for the processing, storage location, and timing of sample collection are listed in the following tables.

4.2.1 HEAD Biofluid Collection Schedule:

Specimen Type	Baseline Visit	18 Month Visit
Whole Blood for RNA	X	X
Serum	X	
Plasma	X	X
DNA	X	X
Whole Blood for banking	X	X

Whole blood is collected in four types of tubes (10ml Serum Tube, 10ml lavender-top EDTA tube, 6ml EDTA tube, and 2.5ml PAXgene™ Blood RNA tube). The 10ml red-top serum tube is processed locally into serum and then aliquoted, frozen at the study site and shipped to NCRAD. The 10ml EDTA tubes are processed locally into plasma and buffy coat fractions. They are then aliquoted, frozen at the study site, and shipped to NCRAD.

The 6ml EDTA tube and PAXgene™ Blood RNA tube are frozen locally without further processing and shipped to NCRAD.

Consent forms must specify that any biological samples and de-identified clinical data may be shared with academic and/or industry collaborators through NCRAD. Recommended consent language can be found on the NCRAD website at: https://ncrad.org/recommended_consent_language.html. A copy of the consent form for each participant should be kept on file by the site investigator.

Frozen samples are to be submitted according to the shipping methods outlined in Section 8.1. Guidelines for the processing, storage location, and timing of sample collection are listed in the following tables.

4.2.2 Biofluid Collection Summary Chart

Draw Order	Collection Tube	Drawn At	Specimen Type	Aliquot Volume	Total Number of Aliquots	Shipping Temperature
1	1 PAXgene™ Blood RNA Collection Tube (2.5 ml)	Baseline	Whole Blood	N/A	N/A	Frozen
		18M				
2	1 Serum Separator (Red-Top) Blood Collection Tube (10ml)	Baseline	Serum	1.5 ml serum aliquots	Up to 4	Frozen
3	5-6 EDTA (Purple-Top) Blood Collection Tubes (10 ml)	Baseline	Plasma	1.5 ml plasma aliquots	Up to 17-21	Frozen
		18M	Buffy Coat	~1.0 ml buffy coat aliquots	Up to 5-6	Frozen
4	1 EDTA (Lavender-Top) Blood Collection Tube (6 ml)	Baseline	Whole blood	N/A	N/A	Frozen
		18M				

4.2.3 Biofluid Collection for Baseline and 18 Month Visits

Sample Type	Tube Type	Study Visits Collecting Biospecimens	Number of Tubes Supplied in Kit	Processing/Aliquoting	Typical # of tubes sent to NCRAD	Ship
Whole Blood for Transcriptome Analysis	**PAXgene™ Blood RNA Collection Tube (2.5 ml)**	**Baseline**	**1**	**N/A**	**1**	**Frozen**
		18M				
Whole blood for isolation for serum	**Serum Determination (Red-Top) Blood Collection Tube (10 ml)**	**Baseline**	**1**	**N/A**	**N/A**	**N/A**
	Serum: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	**Baseline**	4 (3 Red Cap, 1 Blue Cap Cryovial)	1.5 ml serum aliquots per 2.0 ml cryovial	Up to 4	Frozen
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	**EDTA (Lavender-Top) Blood Collection Tube (10 ml)**	**Baseline**	**5**	**N/A**	**N/A**	**N/A**
		18M	**6**	**N/A**	**N/A**	**N/A**
	PLASMA: 2.0 ml cryovials with purple cap (residual volume placed in 2.0 ml cryovial with blue cap)	Baseline	17 (16 Lavender Cap, 1 Blue Cap Cryovial)	1.5 ml plasma aliquots per 2.0 ml cryovial	Up to 17	Frozen
		18M	21 (20 Lavender Cap, 1 Blue Cap Cryovial)	1.5 ml plasma aliquots per 2.0 ml cryovial	Up to 21	Frozen
	BUFFY COAT: 2.0 ml cryovial	Baseline	5 (5 Gray Cap Cryovials)	1 ml buffy coat aliquots per 2.0 ml cryovial	5	Frozen
		18M	6 (6 Gray Cap Cryovials)	1 ml buffy coat aliquots per 2.0 ml cryovial	6	Frozen
Whole blood for future analysis	**EDTA (Lavender-Top) Blood Collection Tube (6 ml)**	**Baseline**	**1**	**N/A**	**1**	**Frozen**
		18M	**1**	**N/A**	**1**	**Frozen**

If a sample is not obtained at a particular visit, it should be recorded in the notes section of the **Biological Sample and Shipment Notification Form (see** Appendix B & Appendix C**).** Submit a copy to NCRAD with a reason provided for the omission and track it as a protocol deviation.

5.0 Specimen Collection Kits, Shipping Kits, and Supplies

NCRAD will provide: 1) Blood sample collection kits for research specimens to be stored at NCRAD, the Blood Supplemental Supply Kit, the Frozen Shipment Supply Kit 2) clinical lab supplies (with the exception of pelleted dry ice and equipment supplies listed in Section 4.1). The provided materials include blood tubes, pipettes, boxes for serum, plasma/buffy coat aliquots, as well as partially completed shipping labels to send materials to NCRAD. Kit Number Labels, HEAD site and PTID Labels, and collection tube and aliquot labels will be provided by NCRAD. Details regarding the blood kits are found in this Manual of Procedures. Collection Tube and Cryovial Labels will be pre-printed with study information specific to the type of sample being drawn. Ensure that all tubes are properly labeled during processing and at the time of shipment according to Section 6.1.

5.1 Specimen Collection Kit Contents

Collection kits contain the following (for each participant) and provide the necessary supplies to collect samples from a given participant. Do not replace or supplement any of the tubes or kit components provided with your own supplies unless you have received approval from the NCRAD Study team to do so. *Please store all kits at room temperature until use.*

HEAD Baseline Blood-Based Kits

Quantity	HEAD Baseline Blood-Based Kit Components
1	Serum red top tube (10 ml)
5	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
1	EDTA (Lavender-Top) Blood Collection Tube (6 ml)
1	PAXgene Blood RNA Tube (2.5ml)
3	2ml Robotic Freezer Cryovials– RED
16	2ml Robotic Freezer Cryovials - PURPLE
2	2ml Robotic Freezer Cryovials - BLUE
5	2ml Robotic Freezer Cryovials - GRAY
5	Disposable graduated transfer pipette (3ml)
1	50ml conical
8	Pre-printed Collection Tube Label
26	Cryovial Label
3	Pre-printed Kit Number Label
8	Labels for handwritten Site and PTID
2	Resealable bubble wrap tube pouches

1	Resealable bag
1	Cryovial box (holds up to 48 cryovials)
1	Plastic Biohazard bag with absorbent sheet (small)

HEAD 18 Month Blood-Based Kits

Quantity	HEAD 18 Month Blood-Based Kit Components
6	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
1	EDTA (Lavender-Top) Blood Collection Tube (6 ml)
1	PAXgene Blood RNA Tube (2.5ml)
20	2ml Robotic Freezer Cryovials - PURPLE
1	2ml Robotic Freezer Cryovials - BLUE
6	2ml Robotic Freezer Cryovials - GRAY
6	Disposable graduated transfer pipette (3ml)
1	50ml conical
8	Pre-printed Collection Tube Label
27	Cryovial Label
3	Pre-printed Kit Number Label
8	Labels for handwritten Site and PTID
2	Resealable bubble wrap tube pouches
1	Resealable bag
1	Cryovial box (holds up to 48 cryovials)
1	Plastic Biohazard bag with absorbent sheet (small)

Blood-Based Supplemental Supply Kit

Quantity	Blood-Based Supplemental Supply Kit Components
2	Serum red top tube (10 ml)
10	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
2	PAXgene Blood RNA Tube (2.5 ml)
2	EDTA (Lavender-Top) Blood Collection Tube (6 ml)
10	Disposable graduated transfer pipette (3ml)
2	50ml Conical Tube
16	Labels for handwritten Site and PTID
4	Bubble wrap tube sleeve for frozen blood tubes
2	Resealable bag
2	Cryovial box (holds up to 48 cryovials)
2	Plastic Biohazard bag with absorbent sheet (large)
32	2ml Robotic Freezer Cryovials - PURPLE
10	2ml Robotic Freezer Cryovials - GRAY
6	2ml Robotic Freezer Cryovials– RED
4	2ml Robotic Freezer Cryovials - BLUE
2	UPS Blue Dry Ice Sticker

2	UN3373 Sticker
2	Fragile Label

HEAD Frozen Blood Shipping Supply Kit

Quantity	Frozen Shipping Kit Components
5	Plastic Biohazard bag with absorbent sheet (large)
1	Resealable bag
1	Shipping box/Styrofoam container
1	UPS Blue Dry Ice Sticker
1	UN3373 Sticker
1	Fragile Label

Individual Supplies

Quantities	Items Available upon request within the NCRAD kit module
By Request	Serum red top tube (10 ml)
By Request	PAXgene Blood RNA Tube (2.5 ml)
By Request	EDTA (Lavender-Top) Blood Collection Tube (10 ml)
By Request	EDTA (Lavender-Top) Blood Collection Tube (6 ml)
By Request	Disposable graduated transfer pipette (3ml)
By Request	50ml conical
By Request	Labels for handwritten Site and PTID
By Request	Bubble wrap tube sleeve for frozen blood tubes
By Request	Resealable bag
By Request	Cryovial box (holds up to 48 cryovials)
By Request	Plastic Biohazard bag with absorbent sheet (large)
By Request	UN3373 sticker
By Request	Dry Ice label
By Request	Fragile labels
By Request	Shipping box
By Request	2ml Robotic Freezer Cryovials - PURPLE
By Request	2ml Robotic Freezer Cryovials - GRAY
By Request	2ml Robotic Freezer Cryovials– RED
By Request	2ml Robotic Freezer Cryovials - BLUE

5.2 Kit Supply to Study Sites

Each site will be responsible for ordering and maintaining a steady supply of kits from NCRAD. We advise sites to keep a supply of each kit type available. Be sure

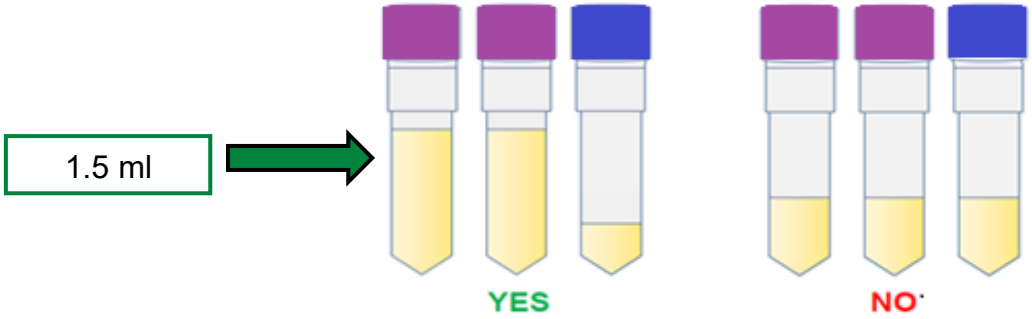
to check your supplies and order additional materials before you run out or supplies expire so you are prepared for study visits. Please go to https://kits.iu.edu/head to request additional kits and follow the prompts to request the desired supplies. Options include ordering a specific number of kits; we are also including the option of simply ordering the desired amount of extra supplies.

Please allow **THREE weeks** for kit orders to be processed and delivered.

5.3 Filling Aliquot Tubes (Serum and Plasma)

In order to ensure that NCRAD receives a sufficient amount of sample for processing and storage, and to avoid cracking of the tubes prior to shipment, each aliquot tube should be filled to the assigned volume after processing is completed (refer to detailed processing instructions for average yield per sample). Over-filled tubes may burst once placed in the freezer, resulting in a loss of sample.

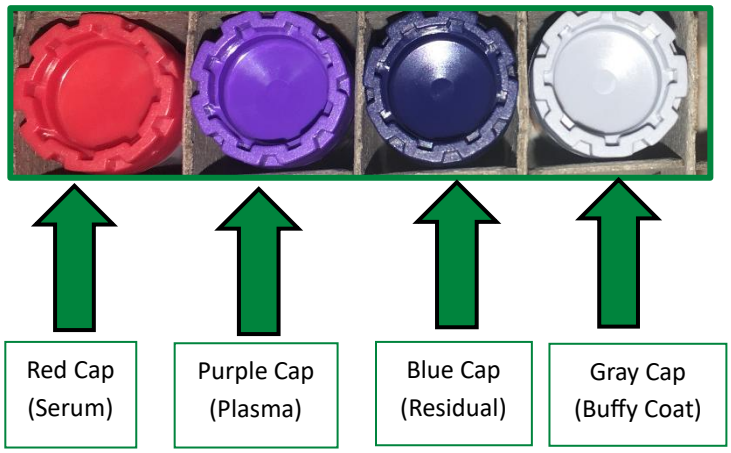
Aliquot the remaining biological material as the residual volume and ship to NCRAD. Essentially, all material should be shipped to NCRAD, ensuring maximum amount in as many cryovials as will allow after processing the sample. For example, if 3.6 ml of sample is obtained, you should fill 2 cryovials each with 1.5 ml, and one additional cryovial with the remaining 0.6 ml.



Please note: It is critical for the integrity of future studies using these samples that study staff note if an aliquot tube contains a residual volume (anything under 1.5 ml). Please highlight that the aliquot contains a small volume by utilizing the blue cryovial cap provided in each kit. Please record the last four digits of the residual aliquot on the Biological Sample and Notification Form. **If there are any unused cryovials, please do not send the empty cryovials to NCRAD. These unused cryovials (ensure labels are removed) can be saved as part of a supplemental supply at your site or the cryovials can be disposed of per your site's requirements.**

To assist in the preparation and aliquoting of samples, colored caps are used for the aliquot tubes. The following chart summarizes the association between cap color and type of aliquot.

Cap Color	Sample Type
Purple	Plasma
Gray	Buffy Coat
Red	Serum
Blue	Residual Aliquot (Plasma or Serum)
White	Plasma (alternative)



6.0 Blood Collection and Processing Procedures

6.1 Labeling Samples

In order to ensure the highest quality samples are collected, it is essential to follow the specific collection and shipment procedures detailed in the following pages. Please read the following instructions first before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.

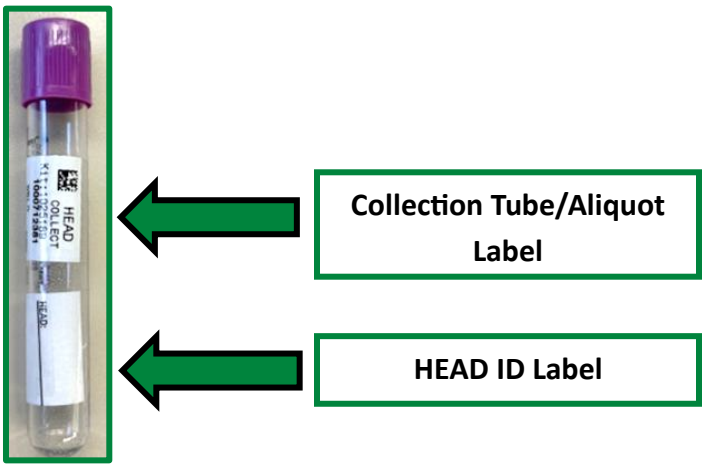
6.1.1 Label Type Summary

1. Kit Number Label
2. HEAD ID Label
3. Collection Tube and Aliquot Label

Kit Number Labels tie together all specimens collected from one participant at one visit. They should be placed on each cryobox, and in the designated location on the Blood Sample and Shipment Notification Forms.

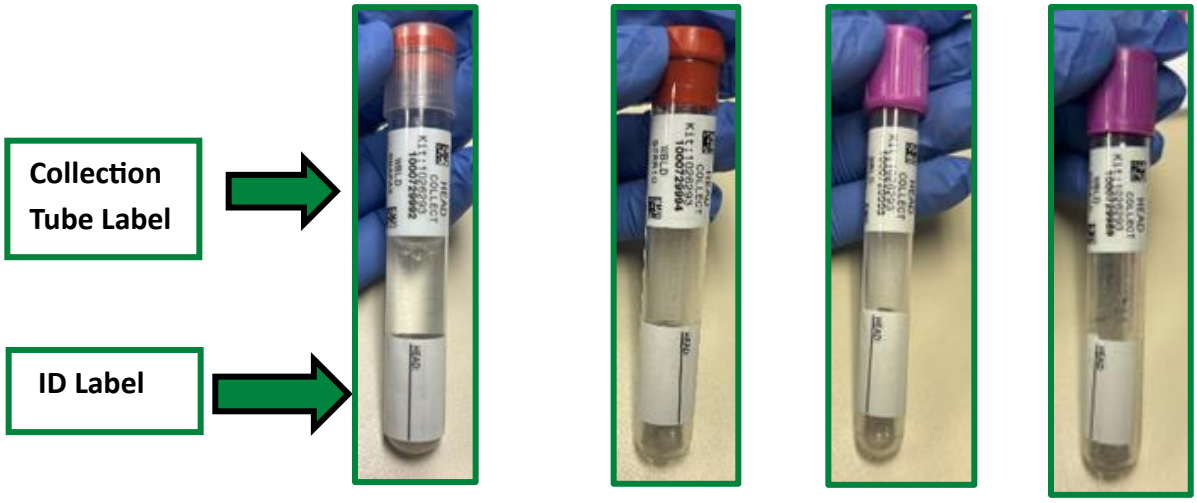
HEAD ID Labels are used to document the individual's unique HEAD ID. Place one label on each blood collection tube.

Place one **Collection Tube and Aliquot Label** on each blood collection tube and Sarstedt tube.



Labeled EDTA (Purple-Top) Blood Collection Tube

Each collection tube will contain two labels: the collection tube label and the HEAD ID Label. Be sure to place labels in the same configuration consistently among tubes, with the barcoded label near the top of the tube and the handwritten HEAD ID label near the bottom of the tube.

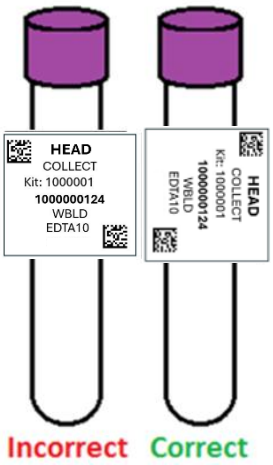


PAXgene™ Blood RNA Collection Tube (2.5 ml)	Serum Determination (Red-Top) Blood Collection Tube (10 ml)	EDTA (Lavender-Top) Blood Collection Tube (6 ml)	EDTA (Lavender-Top) Blood Collection Tube (10 ml)

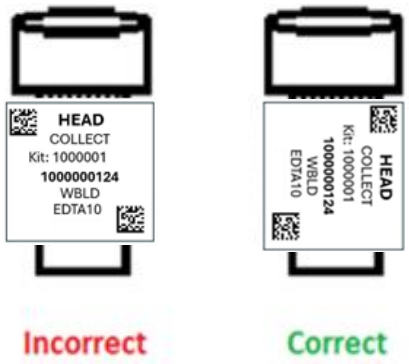
In order to ensure the label adheres properly and remains on the tube, please follow these instructions:

- Place Collection Tube and Aliquot Labels on **ALL** collection tubes and cryovials **BEFORE** sample collection. This should help to ensure the label properly adheres to the tube before exposure to moisture or different temperatures.
- Using a fine point permanent marker, fill-in and place the HEAD ID Labels on the EDTA (purple-top) tubes **BEFORE** sample collection. These labels are placed on collection tubes in addition to the Collection Tube Label.
- The Collection Tube Labels contain a 2D barcode on the left-hand and bottom right-hand side of the label.
- Place label **horizontally** on the tube (wrapped around sideways if the tube is upright) with barcode toward the tube cap.

Take a moment to ensure the label is **completely adhered** to each tube. It may be helpful to roll the tube between your fingers after applying the label. The following pictures show the correct orientation of the labels on the collection tubes and cryovials.



Collection Tube Label Diagram



Aliquot Label Diagram

6.2 2.5 ml PAXgene™ Blood RNA Tube

Whole Blood Collection for Isolation of RNA: 2.5 ml PAXgene™ Blood RNA Tube

1. **Inspect tube expiration date and confirm tube is not expired.**

2. Place filled-out Site and HEAD ID Label and Collection and Aliquot "**Whole Blood**" Tube Label on the PAXgene™ tube (2.5 ml) prior to blood draw; no processing is required for this tube**. <u>The single tube is to be shipped to NCRAD frozen, without processing at the collection site.</u>**

3. Using a blood collection set and a holder, collect blood into the **PAXgene™ Blood RNA Tube (2.5 ml)** using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:
   a. Place participant's arm in a downward position.
   b. Hold tube in a vertical position, below the participant's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into last collection tube.
   d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.

4. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the**

tube from the holder. The PAXgene™ Blood RNA Tube (2.5 ml) with its vacuum is designed to draw 2.5 ml of blood into the tube.

5. **Immediately after blood collection, gently invert/mix (180 degree turns) the PAXgene™ Blood RNA Tube (2.5 ml) 8 – 10 times**.

6. Place the PAXgene™ Blood RNA tube (2.5 ml) upright in a **WIRE** rack and transfer the PAXgene™ Blood RNA tube (2.5 ml) to a **-80°C freezer.** Keep the **PAXgene™ Blood RNA Tube (2.5 ml) in -80°C freezer** for storage until you ship on pelleted dry ice to NCRAD. Complete remainder of the Biological Sample and Shipment Notification Form (Appendix B).

RNA Preparation (2.5ml PAXgene™ Tube)

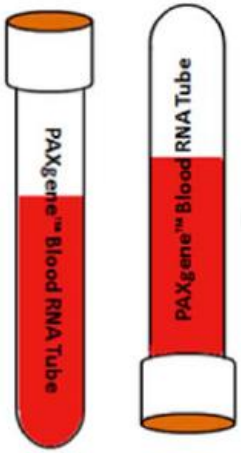
Step One

- **Inspect tube expiration date and confirm tube is not expired.**
- Store tubes at room temperature.
- Label tubes with pre-printed labels prior to blood draw.

Step Two

- Collect blood in PAXgene™ tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

- Store tubes at -80°C in a wire rack until shipment.

6.3 Serum Determination (Red-Top) Tube (10 ml) for Serum

Whole Blood Collection for Isolation of Serum: Serum Determination (Red-Top) Tube (10 ml) (for processing of serum aliquots).

1. **Inspect tube expiration date and confirm tube is not expired.**

2. Store empty plain red-top serum blood collection tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use.

3. Set centrifuge to 4°C to pre-chill before use. Please note that the centrifuge could take 30 minutes to chill completely.

4. Place completed HEAD ID Label and **SERUM** Collection Tube Label on the Plain Red-Top Serum Blood Collection Tube. Place pre-printed **SERUM** Cryovial Labels on the three 2 ml cryovials with red caps and one 2 ml cryovial with blue cap (if necessary, for residual).

5. Using a blood collection set and a holder, collect blood into **Plain Red-Top Serum Blood Collection Tubes (10 ml)** using your institution's recommended procedure for standard venipuncture technique. **The following techniques shall be used to prevent possible backflow:**
   a. Place participant's arm in a downward position.
   b. Hold tube in a vertical position, below the participant's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into last collection tube.
   d. Make sure tube additives do not touch the stopper or the end of the needle during venipuncture.

6. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into each tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
   a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional Serum tube at this time. Process blood obtained in existing Serum tube.

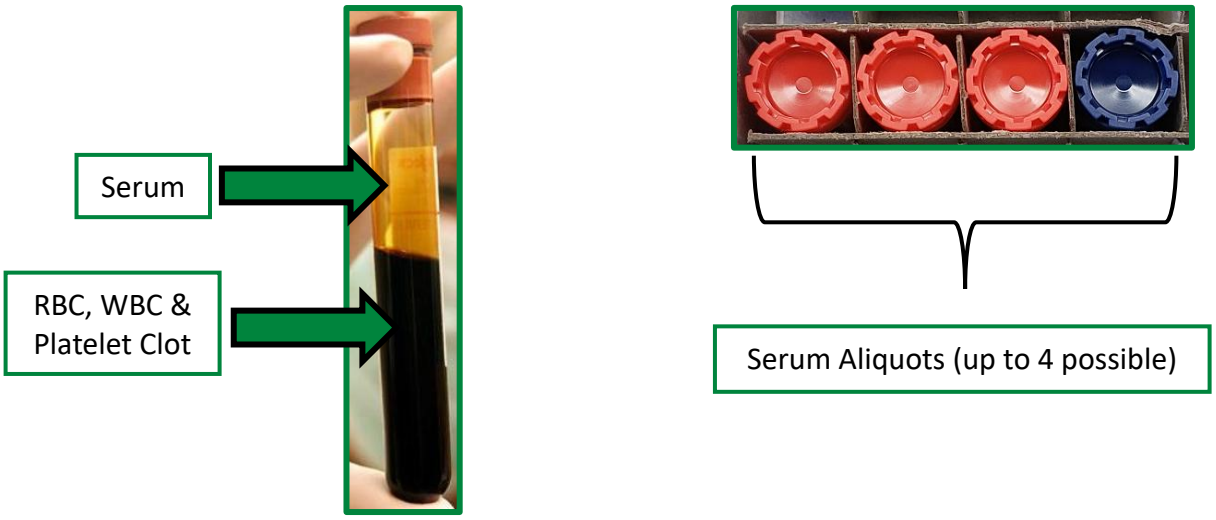
7. Immediately after blood collection, gently invert/mix (180 degree turns) each tube 5 times.

8. **Allow blood to clot at room temperature by placing it upright in a vertical position in a tube rack for 30 minutes.** If sample is not clotted allow it to set up to 60 minutes to clot.

9. After 30 minutes of clotting, centrifuge the collection tube for 10 minutes at 2000 x g at 4°C. **It is critical that the tube be centrifuged at the appropriate speed to ensure proper serum separation (see worksheet in Appendix A to calculate RPM)**
    a. Equivalent rpm for spin at 2000 x g
    b. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form Appendix B.
    c. Record time aliquoted on the Biological Sample Shipment and Notification Form.

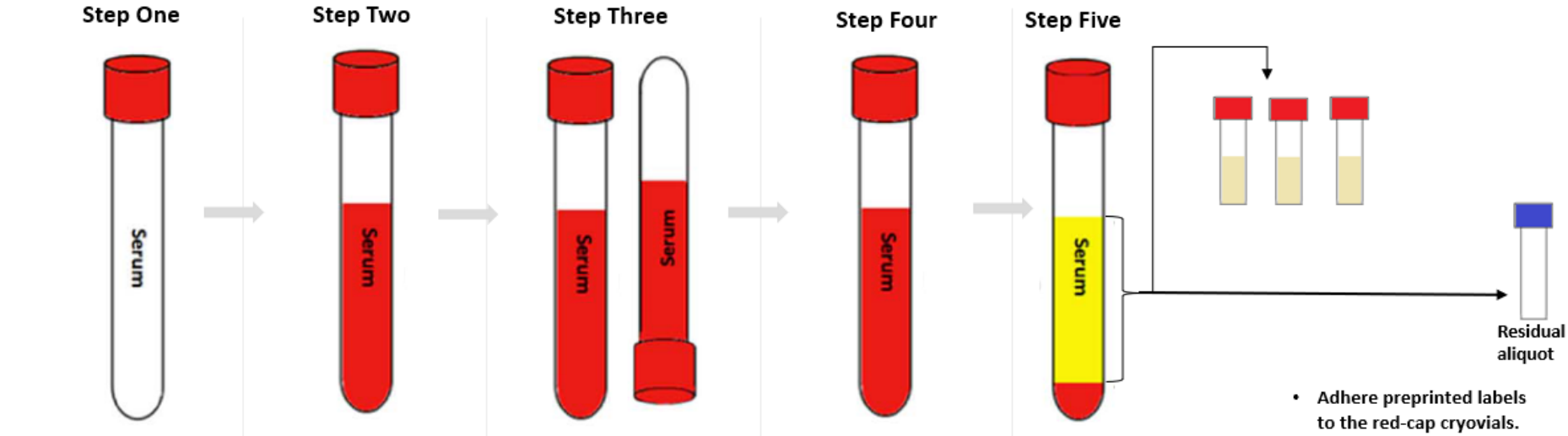
10. Remove the serum by tilting the tube a placing the pipette tip along the lower side of the wall without agitating the packed red blood cells at the bottom of the collection tube.

11. Transfer serum into the pre-labeled cryovials with red caps. The serum tube should yield, on average, 4-5 ml of serum. Aliquot 1.5 ml serum into each cryovial. Be sure to only place **serum** in cryovials with red caps and labeled with **SERUM** labels. Place residual serum (<1.5 ml) in the blue-capped cryovial. **If a residual aliquot (<1.5 ml) is created, document the sample number and volume on the Biological Sample and Shipment Notification Form.**



12. Place the labeled cryovials in the 48 cell cryobox and place on pelleted dry ice. Transfer to -80°C Freezer when possible. Store all samples at -80°C until shipped to NCRAD on pelleted dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample and Shipment Notification Form.

Serum Preparation (10ml Red Top Tube)



Step One

- **Inspect tube expiration date and confirm tube is not expired.**
- Store tubes at room temperature.
- Label tubes and cryovials with pre-printed subject labels prior to blood draw.

Step Two

- Collect blood in Serum Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three

- Immediately after blood draw, invert tube 5 times to mix samples.

Step Four

- Allow blood to clot for 30 minutes.
- Within 60 minutes of blood draw, centrifuge samples at 2000 x g for 10 minutes at 4°C.

Step Five

- Must be spun, aliquoted, and stored in -80°C freezer within 2 hours of collection.
- Adhere preprinted labels to the red-cap cryovials.
- Aliquot 1.5 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number and volume on Sample Notification Form.
- Store plasma aliquots at -80°C until shipment.

6.4 Baseline EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Plasma and Buffy Coat

Whole Blood Collection for Isolation of Plasma and Buffy Coat: EDTA (Lavender-Top) Blood Collection Tube (10 ml) (for processing of plasma aliquots and buffy coat aliquot)

1. **Inspect tube expiration date and confirm tube is not expired.**

2. Store empty EDTA tubes at room temperature, 64□F - 77□F (18 □C – 25 □C) before use. Check expiration dates on all collection tubes before visit.

3. Set centrifuge to 4°C to pre-chill before use.

4. Place completed HEAD ID Label and pre-printed "**PLASMA**" Collection Tube Label on the lavender-top EDTA tubes. Place pre-printed "**PLASMA**" Cryovial Labels on the (16) 2.0 ml cryovials with purple caps and (1) 2.0 ml cryovial with blue cap (if necessary, for residual). Place pre-printed "**BUFFY COAT**" Cryovial Label on the (5) 2.0 ml cryovials with gray caps.

5. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-Top) Blood Collection Tubes (10 ml)** using your institution's recommended procedure for standard venipuncture technique. **The following techniques shall be used to prevent possible backflow:**
    a. Place participant's arm in a downward position.
    b. Hold tube in a vertical position, below the participant's arm during blood collection.
    c. Release tourniquet as soon as blood starts to flow into last collection tube.
    d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

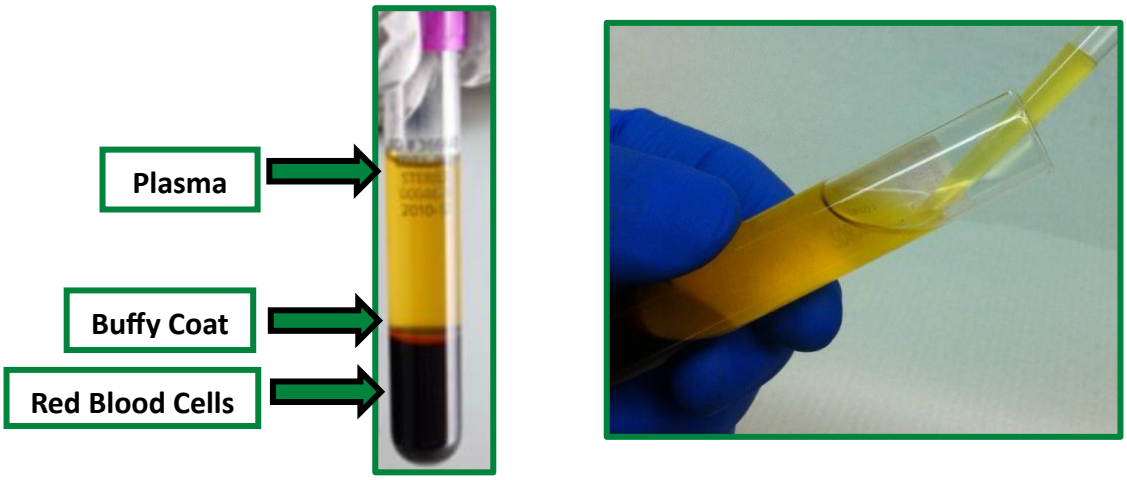
6. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
    a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.

7. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8-10 times.

8. Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.

9. Centrifuge balanced tubes for 10 minutes at 2000 x g at 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix B to calculate equivalent RPM for spin at 2000 x g).**
   a. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
   b. Record original volume drawn for each tube in spaces provided on the Biological Sample Shipment and Notification Form.
   c. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
   d. Record time aliquoted on the Biological Sample Shipment and Notification Form.

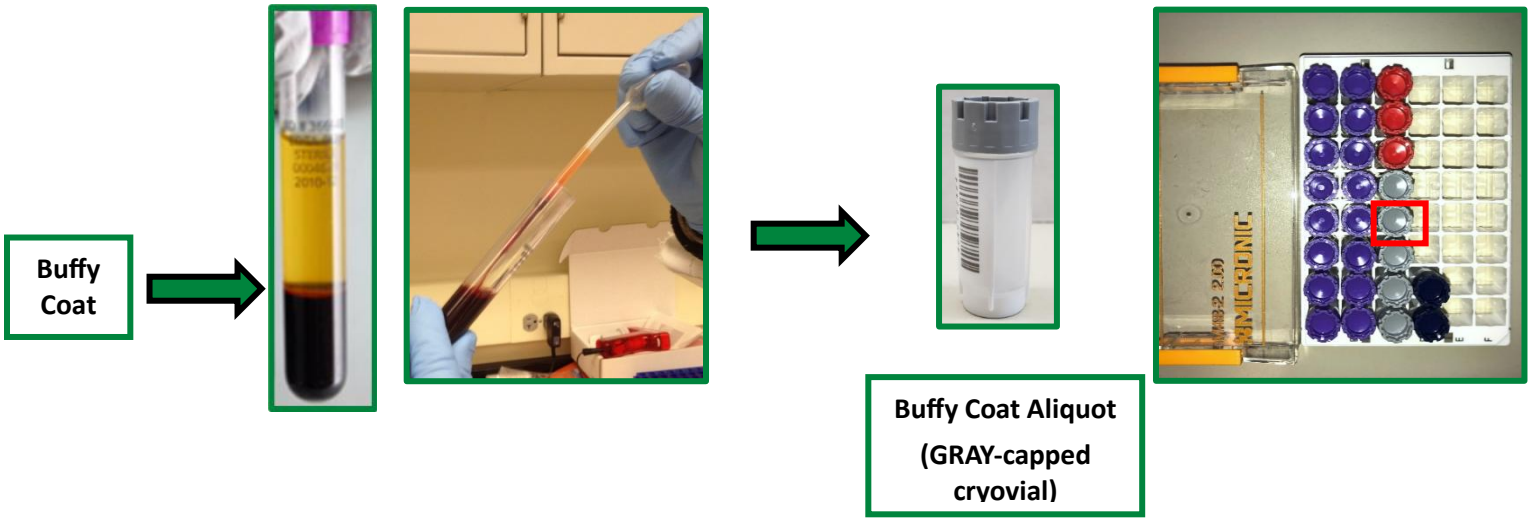
10. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall being careful not to agitate the packed red blood cells at the bottom of the collection tube.



11. Each EDTA tube should yield, on average, 4-5 ml of plasma. Transfer plasma from all EDTA tubes into the 50 ml conical tube and gently invert 3 times. **When pipetting plasma from the EDTA tube into the 50 ml conical tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.** Aliquot 1.5 ml plasma per cryovial. Be sure to only place **plasma** in cryovials with purple caps and labeled with **PLASMA** labels**.** Place residual plasma (<1.5 ml) in the blue-capped cryovial. **If a residual aliquot (<1.5 ml) is created, document the specimen number and volume on the Biological Sample and Shipment Notification Form.**

12. Place the labeled cryovials in the 48-slot cryovial box and place on pelleted dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on pelleted dry ice. Record time aliquots placed in freezer and storage temperature of freezer on Biological Sample Shipment and Notification Form.

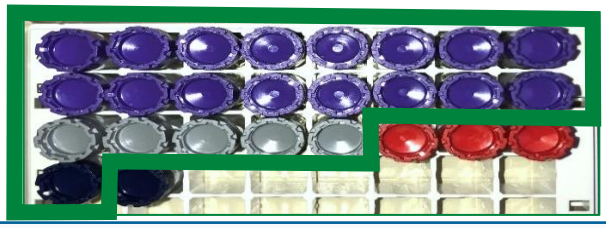
After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tubes (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into labeled cryovials with gray caps using a micropipette. Aliquot each buffy coat into a separate cryovial. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovials with the gray caps and "BUFFY COAT" label.



13. Dispose of tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.

14. Record the specimen number and volumes of the EDTA tubes and corresponding buffy coat samples on the Biological Sample Shipment and Notification Form.

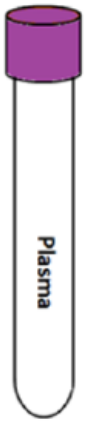
15. Place the labeled cryovials in the 48-slot cryovial box and place on pelleted dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on pelleted dry ice



Plasma Aliquots (up to 17 possible) and Buffy Coat (5)

Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube x 5)

Step One

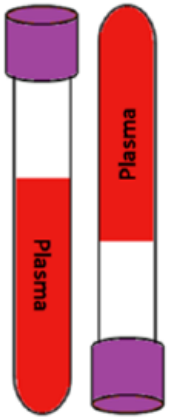


- **Inspect tube expiration date and confirm tube is not expired.**
- Store tubes at room temperature.
- Label tubes with preprinted labels prior to blood draw.

Step Two

- Collect blood in Plasma Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



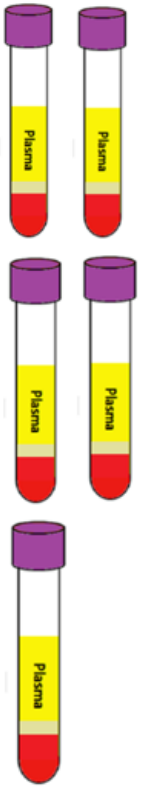
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

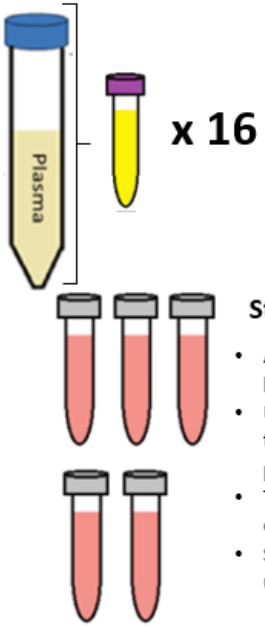
- Place thoroughly mixed tube on wet ice until centrifugation begins.

Step Five

- Preferably within 30 minutes of blood draw, centrifuge samples at 2000 x g at 4°C for 10 minutes (swinging bucket rotor required).
- Samples need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.

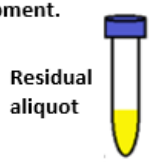


- Pool all plasma from the 5 EDTA tubes into a 50ml conical tube and invert gently 3 times to mix the plasma.



Step Six

- Adhere preprinted labels to the clear cap cryovials with lavender stickers
- Aliquot 1.5 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number and volume on Sample Notification Form.
- Store plasma aliquots at -80°C until shipment.



Step Seven

- Adhere preprinted labels to the blue cap cryovial.
- Using a clean pipette tip, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat into the cryovial tube.
- Store buffy coat aliquot at -80°C until shipment.

6.5 18 Month EDTA (Lavender-Top) Blood Collection Tube (10 ml) for Plasma and Buffy Coat

Whole Blood Collection for Isolation of Plasma and Buffy Coat: EDTA (Lavender-Top) Blood Collection Tube (10 ml) (for processing of plasma aliquots and buffy coat aliquot)

1. **Inspect tube expiration date and confirm tube is not expired.**

2. Store empty EDTA tubes at room temperature, 64°F - 77°F (18 °C – 25 °C) before use. Check expiration dates on all collection tubes before visit.

3. Set centrifuge to 4°C to pre-chill before use.

4. Place completed HEAD ID Label and pre-printed "**PLASMA**" Collection Tube Label on the lavender-top EDTA tubes. Place pre-printed "**PLASMA**" Cryovial Labels on the (20) 2.0 ml cryovials with purple caps and (1) 2.0 ml cryovial with blue cap (if necessary, for residual). Place pre-printed "**BUFFY COAT**" Cryovial Label on the (6) 2.0 ml cryovials with gray caps.

5. Using a blood collection set and a holder, collect blood into the **EDTA (Lavender-Top) Blood Collection Tubes (10 ml)** using your institution's recommended procedure for standard venipuncture technique. **The following techniques shall be used to prevent possible backflow:**
   a. Place participant's arm in a downward position.
   b. Hold tube in a vertical position, below the participant's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into last collection tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

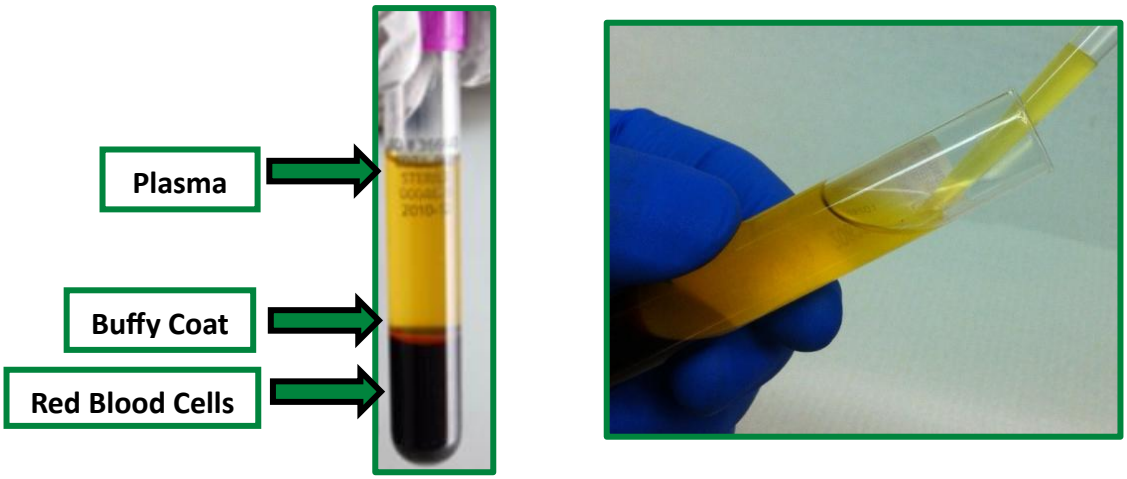
6. Allow at least 10 seconds for a complete blood draw to take place in each tube. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.** The tube with its vacuum is designed to draw 10 ml of blood into the tube.
   a. If complications arise during the blood draw, please note the difficulties on the 'Biological Sample and Shipment Notification Form'. Do not attempt to draw an additional EDTA tube at this time. Process blood obtained in existing EDTA tube.

7. Immediately after blood collection, gently invert/mix (180 degree turns) the EDTA tube 8-10 times.

8. Immediately after inverting the EDTA tube, place it on wet ice until centrifugation begins.

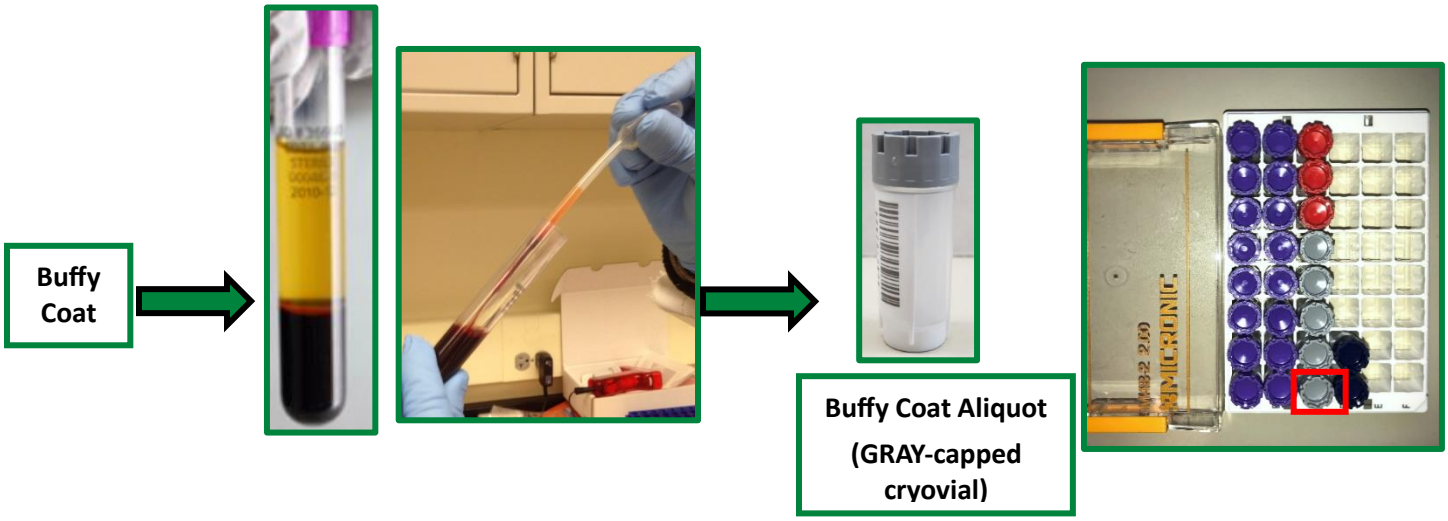
9. Centrifuge balanced tubes for 10 minutes at 2000 x g and 4°C. **It is critical that the tubes be centrifuged at the appropriate speed and temperature to ensure proper plasma separation (see worksheet in Appendix A to calculate RPM.)**
    a. While centrifuging, remember to record all times, temperatures and spin rates on the Biological Sample and Shipment Notification Form.
    b. Record original volume drawn for each tube in spaces provided on the Biological Sample Shipment and Notification Form.
    c. Plasma samples need to be spun, aliquoted, and placed in the freezer within 2 hours from the time of collection.
    d. Record time aliquoted on the Biological Sample Shipment and Notification Form.

10. Remove the plasma by tilting the tube and placing the pipette tip along the lower side of the wall being careful not to agitate the packed red blood cells at the bottom of the collection tube.



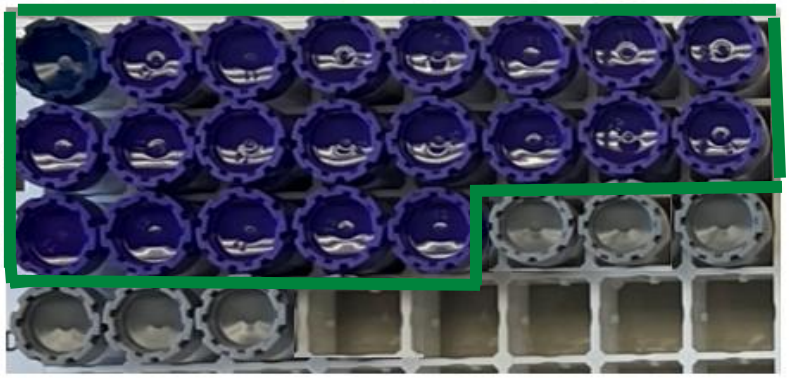
11. Each EDTA tube should yield, on average, 4-5 ml of plasma. Transfer plasma from all EDTA tubes into the 50 ml conical tube and gently invert 3 times. **When pipetting plasma from the EDTA tube into the 50 ml conical tube, be very careful to pipette the plasma top layer only, leaving the buffy coat and the red blood cell layers untouched.** Aliquot 1.5 ml plasma per cryovial. Be sure to only place **plasma** in cryovials with purple caps and labeled with **PLASMA** labels**.** Place residual plasma (<1.5 ml) in the blue-capped cryovial. **If a residual aliquot (<1.5 ml) is created, document the specimen number and volume on the Biological Sample and Shipment Notification Form.**

12. After plasma has been removed from the EDTA (Lavender-Top) Blood Collection Tubes (10 ml), aliquot buffy coat layer (in the top layer of cells, the buffy coat is mixed with RBCs-see figure) into labeled cryovials with gray caps using a micropipette. Aliquot each buffy coat into a separate cryovial. The buffy coat aliquot is expected to have a reddish color from the RBCs. Be sure to place buffy coat into cryovials with the gray caps and "**BUFFY COAT**" label.



13. Dispose of tube with red blood cell pellet according to your site's guidelines for disposing of biomedical waste.

14. Place the labeled cryovials in the 48-slot cryovial box and place on pelleted dry ice. Transfer to **-80°C Freezer when possible**. Store all samples at **-80°C until shipped** to NCRAD on pelleted dry ice.



Plasma (up to 20 possible), Buffy Coat (6), and Plasma residual (1) Aliquots

Plasma and Buffy Coat Preparation (10ml Lavender-Top Tube x 6)

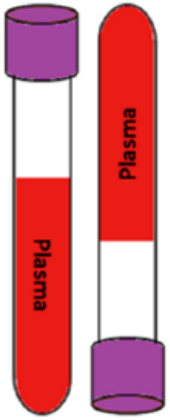
Step One

- **Inspect tube expiration date and confirm tube is not expired.**
- Store tubes at room temperature.
- Label tubes with preprinted labels prior to blood draw.

Step Two

- Collect blood in Plasma Tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



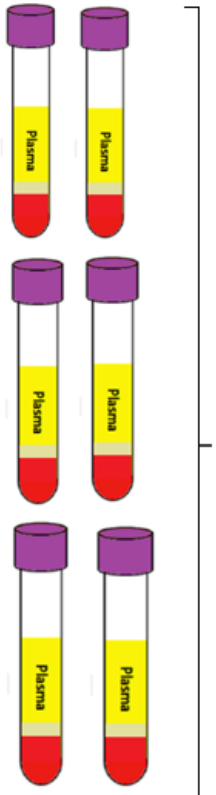
- Immediately after blood draw, invert tubes 8-10 times to mix samples.

Step Four

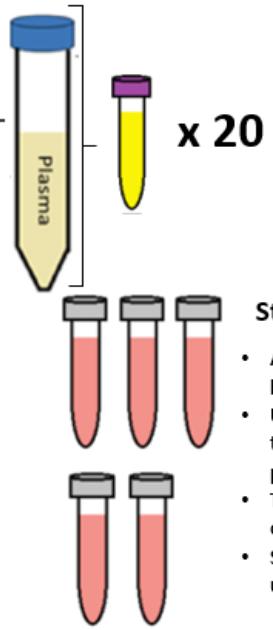
- Place thoroughly mixed tube on wet ice until centrifugation begins.

Step Five

- Preferably within 30 minutes of blood draw, centrifuge samples at 2000 x g at 4°C for 10 minutes (swinging bucket rotor required).
- Samples need to be spun, aliquoted, and in the freezer within 2 hours from the time of collection.



- Pool all plasma from the 5 EDTA tubes into a 50ml conical tube and invert gently 3 times to mix the plasma.



Step Six

- Adhere preprinted labels to the clear cap cryovials with lavender stickers
- Aliquot 1.5 ml into each cryovial tube.
- If a residual aliquot is created, document specimen number and volume on Sample Notification Form.
- Store plasma aliquots at -80°C until shipment.

Residual aliquot

Step Seven

- Adhere preprinted labels to the blue cap cryovial.
- Using a clean pipette tip, collect the buffy coat (may have residual plasma and some RBCs included).
- Transfer the buffy coat into the cryovial tube.
- Store buffy coat aliquot at -80°C until shipment.

6.6 EDTA (Lavender-Top) Blood Collection Tube (6ml)

1. **Inspect tube expiration date and confirm tube is not expired**.

2. Place completed PT ID Label and pre-printed "**WBLD**" Collection Tube Label on the 6ml lavender-top EDTA tube.

3. Using a blood collection set and a holder, collect whole blood into the 6 ml lavender top whole blood tube using your institution's recommended procedure for standard venipuncture technique.

   The following techniques shall be used to prevent possible backflow:

   a. Place participant's arm in a downward position.
   b. Hold tube in a vertical position, below the participant's arm during blood collection.
   c. Release tourniquet as soon as blood starts to flow into last collection tube.
   d. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

4. Invert the tube gently 3 times.

5. Transfer the tube immediately to a **-80°C Freezer.** The sample should be frozen and stored **UPRIGHT** in a WIRE or PLASTIC type test tube rack (DO NOT use a solid Styrofoam test tube holder).

Whole Blood Preparation (6 mL Lavender-Top Tube)

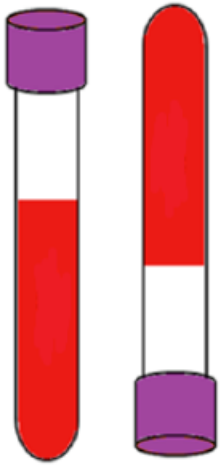
Step One

- Inspect tube expiration date and confirm tube is not expired.
- Store tubes at room temperature.
- Label tubes with pre-printed subject labels prior to blood draw.

Step Two

- Collect blood in tube allowing blood to flow for 10 seconds and ensuring blood flow has stopped.

Step Three



- Immediately after blood draw, invert tube 3 times to mix sample.

Step Four

- Immediately after inversion, freeze the sample in an -80°C freezer until ready to ship.

7.0 Incomplete or Difficult Blood Draws

*****Important Note*****
If challenges arise during the blood draw process, it is advised that the phlebotomist discontinue the draw. Attempt to process and submit any blood-based specimens that have already been collected to NCRAD.

Situations may arise that prevent study coordinators from obtaining the total amount scheduled for biofluids. In these situations, please follow the below steps:

1. If the biofluids at a scheduled visit **are partially** collected:
    a. Attempt to process and submit any samples that were able to be collected during the visit.
    b. Document difficulties on the 'Biological Sample and Shipment Notification Form' prior to submission to NCRAD.
        i. Indicate blood draw difficulties at the bottom of the 'Biological Sample and Shipment Notification Form' within the "Notes" section.
        i. Complete the 'Biological Sample and Shipment Notification Form' with tube volume approximations and number of aliquots created.
    c. Contact a NCRAD coordinator and alert them of the challenging blood draw.

8.0 Packaging and Shipping Instructions

ALL study personnel responsible for shipping should be certified in biofluid shipping (i.e. IATA certification). If not available at your institution, please contact NCRAD with questions and information regarding resources.

8.1 Frozen Packaging Information

FROZEN SAMPLES MUST BE SHIPPED MONDAY-WEDNESDAY ONLY!

Sample Type	Tube Type	Tubes to NCRAD	Ship
Whole blood for transcriptome analysis	PAXgene™ Blood RNA Collection Tube (2.5 ml) for RNA	**1**	Frozen
Whole blood for isolation for serum	SERUM: 2.0 ml cryovials with red cap (residual volume placed in 2.0 ml cryovial with blue cap)	Up to 4 (baseline)	Frozen
Whole blood for isolation of plasma & buffy coat (for DNA extraction)	PLASMA: 2.0 ml cryovials with purple cap (residual volume placed in 2.0 ml cryovial with blue cap)	Up to 17 (baseline) Up to 21 (18 Month)	Frozen
	BUFFY COAT: 2.0 ml cryovial	5 (baseline) 6 (18 Month)	Frozen
Whole blood for future analysis	EDTA (Lavender-Top) Blood Collection Tube (6 ml)	1	Frozen

The most important issue for shipping is to maintain the temperature of the samples. The frozen samples must never thaw; not even the outside of the tubes should be allowed to defrost. This is best accomplished by making sure the Styrofoam container is filled completely with pelleted dry ice.

Large Frozen Shipper – fits 5 48-slot cryoboxes and ~45 lbs.. dry ice

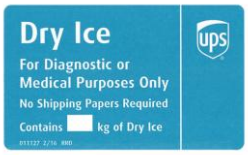
Small Shipper – fits up to 2 48-slot cryoboxes and ~10

Specimens being shipped to NCRAD should be considered as Category B UN3373 specimens and as such must be tripled packaged and compliant with IATA Packing Instructions 650. *See the Latest Edition of the IATA Regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

Packing and Labeling Guidelines

- The primary receptacle (cryovial) must be leak proof and must not contain more than 1L total.
- The secondary packaging (biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material should be of sufficient quantity in order to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest of specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible Person
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373
  - ✓ UPS Dry Ice label and net weight of pelleted dry ice contained

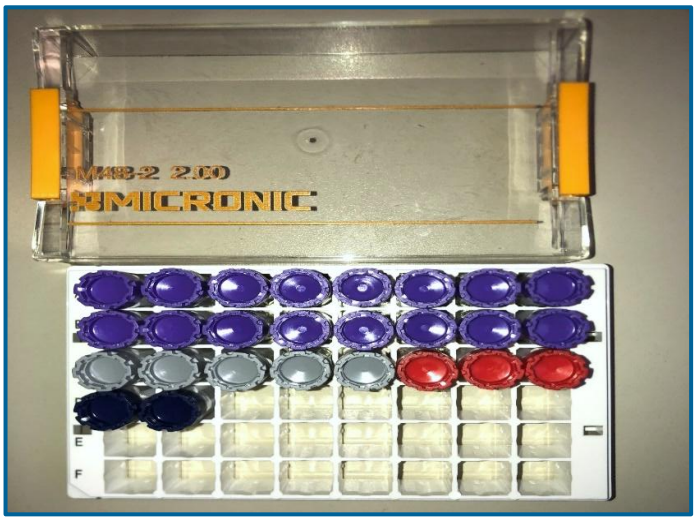


8.1.1 NCRAD Packaging Instructions -Frozen Packaging Instructions

1. If possible, hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off. If storage in a -80°C freezer until UPS pick-up is not possible, package samples no more than 4 hours before the expected pick-up time.

2. Notify NCRAD of shipment by emailing NCRAD coordinators at alzstudy@iu.edu. Attach the following to the email:
    a. Completed Sample Form (Appendix C) to the email notification (email NCRAD coordinator prior to shipment to receive sample form).
    b. If email is unavailable please call NCRAD and do not ship until you've contacted and notified NCRAD coordinators about the shipment in advance.

3. Place the cryovial boxes containing frozen samples into a biohazard bag.
    a. Each 48-slot cryobox will hold approximately 26 cryovial samples (**27 for 18 Month visit**). Place serum, plasma and buffy coat within one cryobox (4 serum, 17 plasma, 5 buffy coat)(**up to 21 plasma and 6 buffy coat for 18 Month visit**) per participant blood draw (see below).
    b. Cryoboxes should contain all of the specimens from the same patient, per time point.
    c. Batch shipping should be performed every (3) three months or when specimens from 5 participants accumulates, whichever is sooner.



One cryobox containing serum, plasma, residuals, and buffy coat

4. Label the outside of the cryoboxes with the appropriate kit number label(s). Place serum, plasma and buffy coat aliquots within one cryobox and place within a biohazard bag. The biohazard bags are large enough to contain one cryobox and 1 frozen blood tube from one subject's visit.
    a. As the cryovial box is placed in the plastic biohazard bag, do NOT remove the absorbent material found in the bag. Seal according to the instructions on the bag.

5. Place approximately 2-3 inches of pelleted dry ice in the bottom of the Styrofoam shipping container.

6. Place the biohazard bags into the provided Styrofoam-lined shipping container on top of the pelleted dry ice. Please ensure that cryovial boxes are placed so the cryovials are upright in the shipping container. **A maximum of 5 cryoboxes may be sent in each shipper.**

Full Shipping Container with
Batched Samples and Dry Ice

7. After the samples have been placed into the shipping container, completely fill the inner Styrofoam with pelleted dry ice pellets to ensure the frozen state of the specimens during transit.

8. Replace the lid on the Styrofoam carton. Place the completed Blood Sample and Shipment Notification Form in the package on top of the Styrofoam lid for each patient specimen, and close and seal the outer cardboard shipping carton with packing tape.

9. Complete the UPS Dry Ice Label with the following information:
    a. Net weight of pelleted dry ice in kg (must match amount on the airbill)
    b. Do not cover any part of this label with other stickers, including preprinted address labels.

10. Apply all provided warning labels and UPS return airbill to the outside of package, taking care not to overlap labels. **Complete the required fields on the UPS Dry Ice label or UPS may reject or return your package.**

IMPORTANT!
Complete the UPS Dry Ice label or UPS may reject or return your package.

11. Use UPS tracking to ensure the delivery occurs as scheduled and is received by NCRAD. Please notify NCRAD by email (alzstudy@iu.edu) that a shipment has been sent and include the UPS tracking number in your email.

12. Hold packaged samples in -80°C freezer until time of UPS pick-up/drop-off.

13. Specimens should be sent to the following address via **UPS Next Day Air**. Frozen shipments should be sent **Monday through Wednesday** to avoid shipping delays on Thursday or Friday. UPS does not replenish dry ice if shipments are delayed or held over during the weekend.

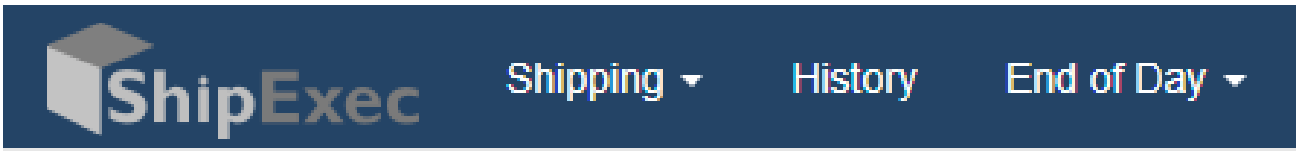
HEAD at NCRAD
Indiana University School of Medicine
351 West 10th Street
TK-217
Indianapolis, IN 46202
Phone: 1-800-526-2839

14. Use UPS tracking to ensure the delivery occurs as scheduled and is received by NCRAD. Please notify NCRAD by email (alzstudy@iu.edu) that a shipment has been sent and include the UPS tracking number in your email.

8.2 Frozen Shipping Instructions

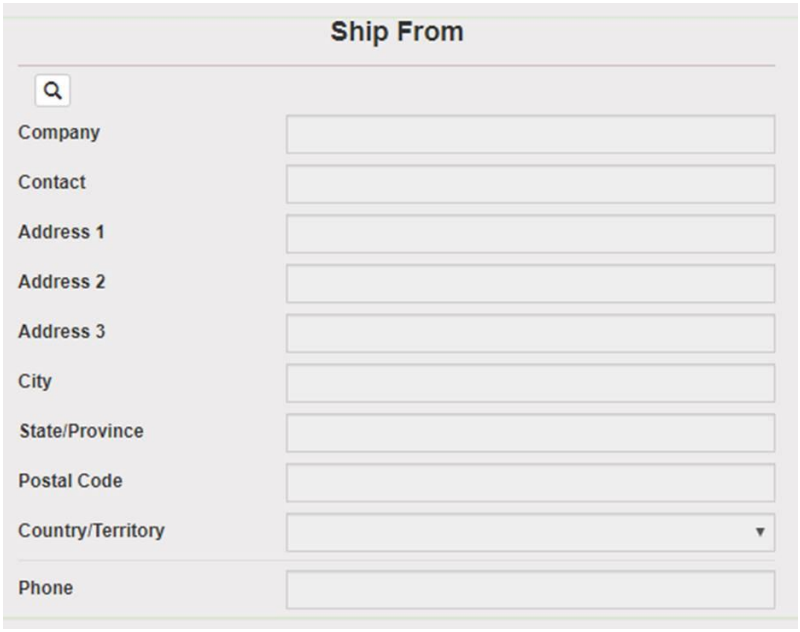
1. Log into the ShipExec Thin Client at kits.iu.edu/UPS.
    a. If a new user or contact needs access, please reach out to your study contact for access.

2. Click “Shipping” at the top of the page and select “Shipping and Rating”



3. Select your study from the “Study Group” drop down on the right side of the main screen. Choosing your study will automatically filter the address book to only addresses within your study.

4. Click on the magnifying glass icon in the “Ship From” section to search for your shipping address.



   a. Search by Company (site), Contact (name), or Address 1 (first line of your site’s street address). Click Search.
   b. Click Select to the left of the correct contact information.

5. Verify that both the shipping information AND study reference are correct for this shipment.
   a. If wrong study contact or study reference, click Reset in the bottom right of the screen to research for the correct information.
6. Enter Package Information
   a. Ambient shipments
      i. Enter the total weight of your package in the “Weight” field and leave the “Dry Ice Weight” field empty.
   b. Frozen shipments
      i. Enter the total weight of your package in the “Weight” field.
      ii. Enter the dry ice weight in the “Dry Ice Weight” field.
      iii. If the “Dry Ice Weight” field is higher than the “Weight” field, you will receive an error message after clicking “Ship” and need to reenter these values.
   c. Click Ship in the bottom right of the page when complete.

7. If your site does not already have a daily UPS pickup, you will need to schedule one
   a. Click the blue Pickup Request button. Enter the earliest pickup time and latest pickup time in 24-hr format.

b. Give a name & phone number of someone who the UPS driver can call if having issues finding the package.
c. Give the Floor and Room Number (if needed) to be as descriptive as possible where this package needs to be picked up from. Click Save.

8. Print the airbill that is automatically downloaded.
    a. To reprint airbill, click History at the top left of the page.
        i. Click Detailed Report from the dropdown menu on the right side of the page.
        ii. Enter tracking number if known. Otherwise, search by ship date. Click Search.
        iii. Click print icon on right side of the tracking number line.

9. Fold airbill and place inside plastic UPS sleeve.

10. Peel the back off of the UPS sleeve and stick the sleeve to the package top. Ensure that sleeve does not cover any warning labels (e.g. pelleted dry ice label) or overlap taped seams.

9.0 Data Queries and Reconciliation

Sample and Shipment Notification forms must be completed on the day that samples are collected because they include information that will be used to reconcile sample collection and receipt, as well as information essential to future analyses.

Data queries or discrepancies with samples shipped and received at NCRAD may result from:

- Incorrect samples collected and shipped
- Damaged or incorrectly prepared samples
- Unlabeled samples, samples labeled with incomplete information, or mislabeled samples
- Discrepant information documented on the Blood Sample and Shipment Notification Form and logged at NCRAD compared to information entered into the NACC database

10.0 APPENDICES LIST

Please complete and return this form by email to the NCRAD Study Team if you have any questions regarding sample processing. The correct RPM will be sent back to you.

Submitter Information

Name: **Site:**

Submitter e-mail:

Centrifuge Information

Please answer the following questions about your centrifuge.

Centrifuge Type

Fixed Angle Rotor: ☐ Swing Bucket Rotor: ☐

Radius of Rotation (mm):

Determine the centrifuge's radius of rotation (in mm) by measuring distance from the center of the centrifuge spindle to the bottom of the device when inserted into the rotor (if measuring a swing bucket rotor, measure to the middle of the bucket).

Calculating RPM from G-Force:

$$RCF = \left(\frac{RPM}{1{,}000}\right)^2 \times r \times 1.118 \quad \Rightarrow \quad RPM = \sqrt{\frac{RCF}{r \times 1.118}} \times 1{,}000$$

RCF = Relative Centrifugal Force (G-Force)
RPM = Rotational Speed (revolutions per minute)
R= Centrifugal radius in mm = distance from the center of the turning axis to the bottom of centrifuge

Comments:

Please send this form to NCRAD Study Coordinator at alzstudy@iu.edu

Appendix B: Baseline Biological Sample and Shipment Notification Form

Please email the form on or prior to the date of shipment.

To: Diont'e Keys Email: alzstudy@iu.edu Phone: 1-800-526-2839

General Information:

From:_____ Tracking #: _____

Phone: _____ Date:_____

Email:_____

Study: HEAD **Visit:** **BASELINE**

Site ID: _____ **PTID:** _____ **Kit #:** KIT BARCODE

Sex (circle one): M F **Year of Birth:** _____

Blood Processing:

Serum (Red-top) Tube (10 mL)			
Time spin started:	_____ [HHMM]	Number of 1.5 mL serum aliquots created **(red cap)**:	_____
Duration of centrifuge:	_____ Minutes	If applicable, volume of residual serum aliquot **(less than 1.5 mL in blue cap)**:	_____ mL
Temp of Centrifuge: ____ °C	Rate of centrifuge: ____ x g	If applicable, specimen number of residual serum aliquot (last four digits):	_____
Original volume drawn:	_____ mL	Time aliquots placed in freezer:	_____ [HHMM]
Time aliquoted:	_____ [HHMM]	Storage temperature in freezer:	_____ °C

Plasma & Buffy Coat (Lavender-top) Tube (10 mL)			
Time spin started:	_____ [HHMM]	Rate of centrifuge:	____ x g
Duration of centrifuge:	_____ Minutes	Time aliquoted:	_____ [HHMM]
Temp of Centrifuge:	____ °C	Number of 1.5 mL plasma aliquots created **(purple cap):**	_____ x 1.5 mL
Original volume drawn - EDTA #1	_____ mL	If applicable, volume of residual plasma aliquot **(less than 1.5 mL in blue cap)**:	_____ mL
Original volume drawn - EDTA #2	_____ mL	If applicable, specimen number of residual plasma aliquot (**Last four digits)**:	_____
Original volume drawn - EDTA #3	_____ mL	Time aliquots placed in freezer:	_____ [HHMM]
Original volume drawn - EDTA #4	_____ mL	Storage temperature in freezer:	_____ °C
Original volume drawn - EDTA #5	_____ mL		
Aliquot volume – Buffy coat #1	_____ mL	Buffy coat aliquot #1 **(last four digits)**:	_____
Aliquot volume – Buffy coat #2	_____ mL	Buffy coat aliquot #2 **(last four digits)**:	_____
Aliquot volume – Buffy coat #3	_____ mL	Buffy coat aliquot #3 **(last four digits)**:	_____
Aliquot volume – Buffy coat #4	_____ mL	Buffy coat aliquot #4 **(last four digits)**:	_____
Aliquot volume – Buffy coat #5	_____ mL	Buffy coat aliquot #5 **(last four digits)**:	_____

Notes:

Appendix C: 18 Month Biological Sample and Shipment Notification Form

Please email the form on or prior to the date of shipment.

To: Diont'e Keys Email: alzstudy@iu.edu Phone: 1-800-526-2839

General Information:

Tracking #: _____

From: _____

Date: _____

Phone: _____

Email: _____

Study: HEAD **Visit:** **18 Month**

Site ID: _____ **PTID:** _____

Kit #: KIT BARCODE

Sex (circle one): M F **Year of Birth:** _____

Blood Collection:

Date Drawn:	[MMDDYY]	Time of Draw:	[HHMM]
Last date participant ate:	[MMDDYY]	Last time participant ate:	[HHMM]

Lavender Top Whole Blood Tube (6 ml)				**RNA (PAXgene™ Tube)**			
Original volume drawn:	_____ mL	Time EDTA tube placed in freezer:	[HHMM]	Original volume drawn:	_____ml	Time PAXgene™ tube placed in freezer:	[HHMM]

Plasma & Buffy Coat (Lavender-top) Tube (10 mL)			
Time spin started:	_____ [HHMM]	Rate of centrifuge:	____ x g
Duration of centrifuge:	_____ Minutes	Time aliquoted:	_____ [HHMM]
Temp of Centrifuge:	____ °C	Number of 1.5 mL plasma aliquots created **(purple cap):**	_____ x 1.5 mL
Original volume drawn - EDTA #1	_____ mL	If applicable, volume of residual plasma aliquot **(less than 1.5 mL in blue cap)**:	_____ mL
Original volume drawn - EDTA #2	_____ mL	If applicable, specimen number of residual plasma aliquot (**Last four digits)**:	_____
Original volume drawn - EDTA #3	_____ mL	Time aliquots placed in freezer:	_____ [HHMM]
Original volume drawn - EDTA #4	_____ mL	Storage temperature in freezer:	_____ °C
Original volume drawn - EDTA #5	_____ mL		
Original volume drawn - EDTA #6	_____ mL		
Aliquot volume – Buffy coat #1	_____ mL	Buffy coat aliquot #1 **(last four digits)**:	_____
Aliquot volume – Buffy coat #2	_____ mL	Buffy coat aliquot #2 **(last four digits)**:	_____
Aliquot volume – Buffy coat #3	_____ mL	Buffy coat aliquot #3 **(last four digits)**:	_____
Aliquot volume – Buffy coat #4	_____ mL	Buffy coat aliquot #4 **(last four digits)**:	_____
Aliquot volume – Buffy coat #5	_____ mL	Buffy coat aliquot #5 **(last four digits)**:	_____
Aliquot volume – Buffy coat #6	_____ mL	Buffy coat aliquot #6 **(last four digits)**:	_____

Notes: